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Valproate and sodium butyrate attenuate manganese-decreased locomotor activity and astrocytic glutamate transporters expression in mice

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ABSTRACT

Manganese (Mn) is an essential trace element, but chronic overexposure to this metal, either environmentally or occupationally may cause manganism, a disease analogous to Parkinson's disease. Inhibitors of histone deacetylases, such as valproic acid (VPA) and sodium butyrate (NaB) exert neuroprotective effects in various animal models of neurological disorders. Thus, the present study investigated whether VPA or NaB prevent Mn-induced neurotoxicity by assessing locomotor activities and expression of astrocytic glutamate transporters, glutamate transporter 1 (GLT-1) and glutamate aspartate transporter (GLAST), in C57BL/6 mice. C57BL/6 mice were pretreated with VPA (200 mg/kg, i.p.) or NaB (1200 mg/kg, i.p.) prior to intranasal instillation of Mn (30 mg/kg) continually for 21 days, followed by open-field and rota-rod behavioral tests and analyses of astrocytic glutamate transporters GLT-1 and GLAST protein/mRNA levels. The results showed that Mn significantly decreased locomotor activity as determined by total distance travelled, stereotypic and ambulatory counts. Mn also significantly decreased rota-rod activity reflecting altered motor coordination. Pretreatment with VPA and NaB with Mn reversed the effects of Mn on the locomotor activity and motor coordination. VPA and NaB also attenuated the Mn-induced decrease in GLT-1 and GLAST mRNA and protein levels in the cerebral cortical and cerebellar regions of mice. These results suggest that VPA and NaB exert protective effects against Mn toxicity seem *in vitro* are also shown *in vivo.* VPA and NaB pretreatment in mice enhancing astrocytic glutamate transporter GLT-1 expression as well as locomotor activities. Future research endeavors are warranted to determine if the therapeutic potential of VPA and NaB is *via* common molecular mechanism, namely, inhibition of histone deacetylases.

1. Introduction

Abnormalities in behavioral and locomotor activities are considered as physiological indicators of neurological disorders, such as Parkinson's disease (PD), epilepsy, Alzheimer's disease (AD) and autism (Maski et al., 2011). Although the molecular mechanisms of these neurological disorders are still not completely understood, their etiology is based on genetic, environmental or gene x environment interactions (Dauncey, 2012; Peres et al., 2016). Genetic abnormalities leading to neurological diseases are associated with rare gene mutations and irregular gene expression (Dauncey, 2012; Karki et al., 2015; Seifert et al., 2006).

Environmental and idiopathic factors also significantly influence the development and progression of neurodegenerative disorders (Peres et al., 2016; Robison et al., 2012). Previous studies have reported that environmental agents may induce toxicity, which can lead to serious brain injury and damage (Dauncey, 2012; Peres et al., 2016). One of the most studied metal toxicants is manganese (Mn), which is an essential trace element, but chronic high level exposures to this metal may cause a neurological disorder referred to as manganism, with similar clinical features to parkinsonism (Peres et al., 2016), characterized by locomotor dysfunction and behavioral abnormality (Peres et al., 2016; Robison et al., 2012). Upon overexposure, Mn accumulates

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preferentially in specific brain regions (i.e. globus pallidus), resulting in motor deficits and neurological disorders (Robison et al., 2012). Several therapeutic approaches have been reported for the treatment of manganism, such as levodopa, antioxidants, plant extracts, iron (Fe)-chelating agents and glutathione precursors (Peres et al., 2016).

Glutamate-mediated excitotoxicity is considered one of the critical mechanisms involved in Mn neurotoxicity (Brouillet et al., 1993). The astrocytic glutamate transporters, glutamate aspartate transporter (GLAST) and glutamate transporter 1 (GLT-1) in rodents (excitatory amino acid transporter 1 (EAAT1) and excitatory amino acid transporter 2 (EAAT2) in humans, respectively) are the main transporters to uptake most of glutamate from synaptic clefts after binding to its postsynaptic receptors to transfer glutamate signals. Accordingly, dysfunction of these transporters is directly associated with glutamate excitotoxicity. In fact, several idiopathic neurodegenerative diseases including PD and manganism are associated with excitotoxicity along with a decrease in the function and expression of the glutamate transporter, GLT-1.

Targeting epigenetic regulation, in particular deacetylation of histone molecules using inhibitors of histone deacetylases (HDACs) for the development of neuroprotective and anti-cancer agents has shown significant progress in recent years. Valproic acid (2-*n*-propylpentanoic acid; VPA) is clinically used as an antiepileptic and anticonvulsant agent exhibiting increasing usage over the years for the treatment of neurological disorders. Interestingly, in addition to its beneficial effect against epilepsy, VPA exerts neuroprotective effects in several animal models of neurodegenerative diseases (Monti and Contestabile, 2009). However, the exact molecular mechanism of its neuroprotective effect remains to be established.

VPA affects cell growth, differentiation and apoptosis, by regulating gene expression at the molecular level, through epigenetic mechanisms as an inhibitor of HDACs (Ximenes et al., 2013). In addition to inhibition of HDACs, VPA can also interfere with multiple regulatory mechanisms such as the glycogen synthase kinase (GSK)3-α and −β, protein kinase B (Akt), extracellular signal-regulated kinases (ERK) and phosphoinositol pathways, tricarboxylic acid (TCA) cycle, and γ-aminobutyric acid (GABA) system. VPA also showed beneficial effects in modulating blood brain barrier (BBB) disruption and brain edema in a rat model of cerebral ischemia (Wang et al., 2011). VPA also exerts anti-inflammatory and antioxidative properties.

Sodium butyrate (NaB), a short chain fatty acid) is an inhibitor of HDACs, and generally derived from the microbial fermentation product of dietary fiber in the colon and thus, inhibits intestinal pathogenic bacteria and maintains gastrointestinal homeostasis (Canani et al., 2011; Kim et al., 2012). NaB, thus, exerts multiple beneficial effects from the intestinal tract to the peripheral tissues (Canani et al., 2011). NaB has been shown to be effective in the prevention and treatment of multiple chronic disorders such as cancer, metabolic syndrome, cardiovascular diseases, and neurodegeneration. Inhibition of HDACs by NaB is related to its epigenetic regulatory effects on gene expression. Due to its modulation on epigenetic mechanisms, NaB has been considered as a specific and efficacious therapeutic strategy for treatment of variety of diseases, including neurodegenerative disorders. In rats, NaB exhibits neuroprotective effects against ischemic stroke by anti-inflammatory effects (Park and Sohrabji, 2016), and antidepressant-like effects (Yamawaki et al., 2012). NaB also exerts neuroprotective effects by anti-apoptotic, −oxidative and −inflammatory effects in a mouse model of cerebral ischemic injury (Sun et al., 2015). NaB improved memory deficits in a mouse model of traumatic brain injury, attributing to increased expression of tight junction-associated proteins such as occludin and ZO-1, and BBB permeability (Li et al., 2016).

Since VPA and NaB exert neuroprotective effects against numerous neurological disorders, not only by HDAC inhibition, but also many other molecular mechanisms, the current study investigated whether VPA and/or NaB attenuate Mn-induced neurotoxicity by assessing neurobehavioral deficits and expression of astrocytic glutamate transporters, GLT-1 and GLAST in mice.

2. Materials and methods

2.1. Experimental animals

All animal protocols were reviewed and approved by the Meharry Medical College Institutional Animal Care and Use Committee (Nashville, TN). Adult male C57BL/6 mice (6–8 weeks old; weight 18–20 g) were purchased from the Jackson Laboratory and housed for one week prior to study (Bar Harbor, ME). The animals were randomly selected, group-housed (4 mice/cage) and maintained on a 12-h light/dark cycle at 25 ± 2 °C and 60–70% relative humidity with food, water and enrichment available *ad libitum* in the Animal Care Facility of Meharry Medical College (Nashville, TN).

2.2. Chemicals and reagents

Manganese chloride ($MnCl_2$, ≥99% purity), valproic acid (VPA, ≥98% purity), and sodium butyrate (NaB, ≥98% purity) were obtained from Sigma-Aldrich (St. Louis, MO). EAAT1 (ab416) and EAAT2 (ab41621) antibodies were obtained from Abcam (Cambridge, MA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH; sc-14007) antibody was acquired from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-rabbit IgG-HRP-conjugated (W4018) secondary antibody was obtained from Promega (Madison, WI).

2.3. Experimental procedure

The number of animals needed for this study (n = 48; 8 animals/group) was determine by power analysis. Mice were randomly separated into control, VPA, Mn, Mn plus VPA, NaB, Mn plus NaB groups, and initial body-weights were recorded. Male mice were used to alleviate any possible effects of female sex hormone estrogens on the modulation of astrocytic glutamate transporters (Dhandapani and Brann, 2007; Lee et al., 2009). Body-weights were recorded on a weekly basis. Mice were treated once daily for 21 consecutive days with 100 μl of intraperitoneal (i.p.) injection of VPA (200 mg/kg), NaB (1200 mg/kg), or saline (NaCl, 0.9%; control). VPA and NaB were diluted in 0.9% saline. After 30 min, Mn plus VPA, Mn plus NaB, and Mn groups received 2 μl of $MnCl_2$ (30 mg/kg) *via* intranasal instillation in the left nostril. Likewise, VPA, NaB and control groups received 2 μl of distilled water in the left nostril. Previously published protocols for Mn intranasal instillation (Kim et al., 2012), VPA (Loscher et al., 1993; Zaky et al., 2014) and NaB dosing and route (Ferrante et al., 2003; Langley et al., 2008) were implemented. During the instillation period the mice were placed under isoflurane-induced anesthesia for 3 min pre- and post-instillation to sedate and prevent expulsion of Mn from the nostril, respectively.

2.4. Open-field test

Behavioral data were collected on day 21 in Seamless Open-field Activity Arenas using Activity Monitor 5 software (Med Associates, Fairfax, VT). Each open-field arena was made of clear Plexiglas, measuring 27.3 cm x 27.3 cm x 20.3 cm, and was covered with a Plexiglas lid containing air holes. The following activity

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